

In the Specification:

Please make the following changes:

On page 2, please amend paragraphs 2 and 3 of the Brief Description of the Drawings as follows:

Fig. 2. RP-HPLC analysis of the reaction mixture containing Ac-Phe-Lys-Gly-NH₂ (0.1 mM), dns-ε-aca-QQIV (0.5 mM), and guinea pig liver transglutaminase (0.02 U/ml) at 25 °C after 12 min. The peaks were confirmed by LC-ESI/MS (Fig. 3) as Ac-Phe-Lys-Gly-NH₂ (a), ⁶N-dansyl-L-lysine (b), dns-ε-aca-(SEQ ID NO: 1)QQIV (c), and product (d).

Fig. 3. LC-ESI/MS analysis of the reaction mixture containing Ac-Phe-Lys-Gly-NH₂ (2.4 mM), dns-ε-aca-(SEQ ID NO: 1)QQIV (0.5 mM) and guinea pig liver transglutaminase (0.02 U/ml) at 25°C after 9 min. M/z 392.2 (M+1): Ac-Phe-Lys-Gly-NH₂ (a); m/z 380.2 (M+1): ⁶N-dansyl-lysine (b); m/z 833.3 (M+1): dns-ε-aca-(SEQ ID NO: 1)QQIV (c); and m/z 1207.4 (M+1): the cross-linking product (d).

On page 3, please amend paragraph 2 as follows:

Fig. 5. Time course of the reaction between various concentrations of DOPA-Phe-Lys-Gly-NH₂ and dns-ε-aca-(SEQ ID NO: 1)QQIV (0.5 mM) catalyzed by guinea pig liver transglutaminase (0.01 U/ml).

On page 8, please amend Table 1 as follows:

Table 1. Peptide sequences and their substrate specificity toward tissue TGase.

Peptide Sequence	$k_{cat}/K_{m,app}$ *
Ac-KG-NH ₂	10.6
FKG-NH ₂	61.6
LKG-NH ₂	48.4
DOPA-KG-NH ₂	----
Ac-FKG-NH ₂	560
Ac-LKG-NH ₂	482
DOPA-FKG-NH ₂	1324
DOPA-LKG-NH ₂	1179
<u>SEQ ID NO: 2</u> Ac-GQQQLG-NH ₂	34.1
<u>SEQ ID NO: 2</u> DOPA-GQQQLG-NH ₂	47.9
<u>SEQ ID NO: 3</u> NH ₂ -GQLKHLEQQEG-NH ₂	47.3

*min⁻¹mM⁻¹. For details see Examples, below.

On page 8, please amend paragraphs 1 and 2 as follows:

More subtle differences were noted in the specificities of the acyl donor peptides, with all three designed Gln peptides exhibiting good substrate properties. It is interesting to note that the specificities of representative short peptides of this invention, Ac-(SEQ ID NO:2)GQQQLG-NH₂ and DOPA-(SEQ ID NO:2)GQQQLG-NH₂, compared favorably to the specificity of NH₂-(SEQ ID NO:3)GQLKHLEQQEG-NH₂, a peptide derived from the repeat motif found in the keratinocyte protein involucrin, which is known to be an excellent substrate for TGase.

The acyl donor and acyl acceptor peptides of this invention can be separately conjugated or coupled with, for example, PEG. Solutions of such polymer-peptide conjugates rapidly form hydrogels in the presence of transglutaminase under physiological and/or appropriate reaction or end-use conditions. The hydrogels of this invention are adhesive, for example, comparable to type I collagen and guinea pig skin. For example, based on the results of substrate specificity studies, DOPA-FKG (acyl acceptor) and Ac-(SEQ ID NO:2)GQQQLG (acyl donor) were selected and separately coupled to a PEG to form PEG-peptide conjugates 1 and 2 shown in Figs. 7-8. The PEG-peptide conjugates were analyzed and purified by RP-HPLC, and their structures confirmed by MALDI TOF-MS analysis.

On page 9, please amend the first full paragraph as follows:

Materials. Unless otherwise provided herein: 4-armed PEG with amine end groups ($M_w = 10k$) was purchased from SunBio PEG Shop. Hydroxyl terminated PEG ($M_w = 4k$) and Sephadex® LH-20 were purchased from Fluka. Rink amide resin (0.6 mmol/g), H-Gly-2-ClTrt resin (0.6 mmol/g), DCC, BOP, HOBT, DIEA, NMP, and protected amino acids were purchased from Advanced ChemTech, KY, USA. Transglutaminase from guinea pig liver, Boc-L-Lys(Boc)-OH, and *N*-Boc-L-DOPA dicyclohexylammonium salt was purchased from Sigma Chemical Company (St. Louis, MO). Acetonitrile was from Burdick and Jackson. TFA was from J. T. Baker. Triethylamine (Et_3N), piperidine and water (HPLC grade) monodansyl cadaverine were purchased from Aldrich Chemical Company (Milwaukee, WI). Dansyl- ϵ -aminocaproyl-Gln-Gln-Ile-Val (dns- ϵ -aca-(SEQ ID NO:1)QQIV) was a gift from Dr. Laszlo Lorand of Northwestern University Medical School and prepared using well-known synthetic techniques.

On page 13, please amend paragraph 1 of Example 2 as follows:

Example 2

Substrate Specificity. Enzymatic reactions were carried out in 50 mM Tris-HCl buffer containing 5 mM $CaCl_2$, 5 mM DTT, 1 mM EDTA, 0.5 mM dns- ϵ -aca-(SEQ ID NO:1)QQIV (or 1 mM monodansyl cadaverine for glutamine peptides), varying amounts of a peptide substrate, and purified guinea pig liver transglutaminase (Sigma) (0.01-0.08 U/ml) in a total volume of 200 μ l of reaction mixture at pH 8.0, 25°C. At predetermined time intervals, aliquots of the reaction mixture were removed and added to an equal volume of 1% trifluoroacetic acid (TFA) in water or 1% TFA in water containing 0.2 mM 6N -dansyl-L-lysine (Sigma) as the internal standard to terminate the reaction. All reaction products were characterized by LC-ESI/MS (Table 3) and quantitatively analyzed by RP-HPLC. Representative results for a few select peptides are shown in

Figures 2-5. Kinetic constants (Table 4a-b and Figure 6) were calculated from two methods: fit of the initial rates versus substrate concentrations to the Michaelis-Menten equation using the SigmaPlot® 2000 program with Enzyme Kinetics Module (SPSS Inc., IL); and graphing of the kinetic data using the direct linear plot. A molecular weight of 90,000 Da was used to calculate the enzyme concentration. (See, Folk, J.E.; Cole, P.W. *J. Biol. Chem.* **1966** *241*, 5518-5525.)

On page 15, please amend Example 3 as follows:

Example 3

Protected Peptide Fragment Synthesis. Based on the results of substrate specificity studies, one lysine peptide sequence, DOPA-Phe-Lys-Gly, and one glutamine peptide sequence, Ac-(SEQ ID NO:2)Gly-Gln-Gln-Gln-Leu-Gly, were chosen and synthesized manually as protected peptide fragments by Fmoc strategy on a H-glycine-2-chlorotrityl resin with the following amino acid side-chain protections: trityl (Gln), Boc (Lys), and Boc (α -NH₂ of DOPA). Protected peptide fragments were obtained by treatment of the resin with 1% TFA in dichloromethane (DCM), and the cleaved peptide sequences were confirmed by MALDI TOF-MS analysis.

On page 16, please amend the first paragraph of Example 6 as follows:

Example 6

Optimization of buffer systems for hydrogel formation. The two peptides, Ac(SEQ ID NO:2)GQQQLG-NH₂ and DOPA-FKG-NH₂, used to illustrate the polymer-peptide conjugates, above, were chosen as a model system for the optimization of buffer systems for rapid cross-linking: Solution 1. 4 mM Ac(SEQ ID NO:2)GQQQLG-NH₂ and 4 mM DOPA-FKG-NH₂, 2.67 mM EDTA in water; Solution 2. Buffer containing

20 mM CaCl₂, and Solution 3. TGase (0.4 U/ml), 1.33 mM EDTA, and 20 mM DTT in water.

On page 19, please amend the second paragraph of Example 10 as follows:

Peptide synthesis. Each of the following tissue transglutaminase (tTG) peptide substrates was synthesized manually using fluorenylmethoxycarbonyl (Fmoc) solid phase synthesis. The lysine substrate, phenylalanine-phenylalanine-lysine-glycine-cysteine-NH₂(SEQ ID NO:4)FFKGC-NH₂), was synthesized using Rink amide resin. It was cleaved from the resin and deprotected using trifluoroacetic acid (TFA), ethanedithiol (EDT), water, and triisopropylsilane (95:2.5:2.5:1). These reagents were removed via rotor evaporation and the peptide was precipitated in ethyl ether. Once the ethyl ether had evaporated, the peptide was dissolved in 2% acetic acid, frozen at -20 °C and lyophilized. The peptide was stored at -20 °C until use. The yield exceeded 90%, with >85% purity (data not shown). The structure was confirmed with matrix assisted laser desorption ionization time of flight mass spectrophotometry (MALDI TOF) and purity was determined using HPLC.

On page 20, please amend the first paragraph as follows:

The glutamine peptide substrate, acetyl-glycine-glutamine-glutamine-glutamine-leucine-glycine (Ac(SEQ ID NO:2)GQQQLG), was synthesized on chlorotrityl resin which facilitated easy cleavage of the protected peptide, AcG(Q(Trtl))₃LG (Trtl=Trityl). The peptide was cleaved with 1% (v/v) TFA in dichloromethane (DCM) and precipitated with water (>90% yield). The peptide was dried and stored over phosphorus pentoxide without further purification. The predicted molecular weight of the peptide was confirmed by MALDI TOF MS and purity determined with HPLC.

On the bottom of page 20 and the top of page 21, please amend the first paragraph of Example 11 as follows:

Example 11

Synthesis of alginate modified with a lysine substrate. A 2% solution of high-mannuronic acid alginate in 0.1 M MES and 0.3M NaCl at pH 6.5 was combined with 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide(Sulfo-NHS) and [N-ε-maleimidocaproic acid] hydrazide (EMCH). The COOH:EDC:sulfo-NHS:EMCH ratio was 8:2:1:2. With reference to Fig. 11, the reaction was performed at room temperature for 2 hours after which the unreacted solutes and byproducts were removed using centrifugal ultrafiltration for 2.5 hours. The lysine substrate, SEQ ID NO:4 FFKGC-NH₂, was then added at equimolar quantity to EDC and the reaction proceeded at room temperature for 3.5 hours. The unreacted peptide was removed by centrifugal ultrafiltration and solvent exchanged to water. The solution was considered pure when the filtrate tested negative for primary amines utilizing the ninhydrin test. The solution was then lyophilized and stored at -20 °C until use.

Finally, please insert pages 1 and 2, corresponding to the sequence listing for the this application, after page 28 of the patent application (after the Abstract of the Invention).